IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Art Unit: 1648
Ashok AMIN	Examiner: Donna C WORTMAN
Appln. No.: 09/925,970	Washington, D.C.
Date Filed: August 10, 2001	Confirmation No. 4363
For: METHOD FOR TREATING HEPATITIS	ATTY.'S DOCKET: AMIN=4A

DECLARATION UNDER 37 CFR 1.132

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Arlington, Virginia 22202

Sir:

I, Steven B. Abramson, M.D., do hereby declare that I am one of the inventors of the above-identified application.

We have discovered that compounds which neutralize the activity of secreted TNF, namely, etanercept, adalimumab and infliximab, bind TNF and block inflammation by inhibiting the downstream effect of TNF.

Hepatitis is an inflammatory disorder which can be caused by viral infections, including Epstein-Barr, cytomegalovirus, and hepatitis A-E viruses. We have discovered that hepatitis can be effectively treated by administering to a patient an effective amount of a compound

that neutralizes the activity of TNF, such as etanercept and infliximab.

Under my direction and control, one patient who suffered from hepatitis C (HCV) was treated with Enbrel. I have also reviewed the case histories of six additional cases similarly treated, which were brought to my attention by other physicians. In all seven cases, Enbrel was use to treat rheumatoid arthritis in these patients who had concomitant and untreated HCV infection. This treatment with Enbrel was begun during a time in which there was concern that TNF blockers might exacerbate chronic hepatitis C infection and, therefore, the response of these patients was unexpected and could not have been predicted based upon the state of medical knowledge at the time (1997-2000). This concern about the use of TNF blockers was reiterated recently in Peterson et al., *Ann Rheum Dis* 2003 62(11):1078-1082, in which the authors stated, "Tumor necrosis factor alpha (TNF alpha) antagonists are effective for the treatment of rheumatoid arthritis, but concerns remain about the safety of these agents in the presence of chronic infections, including hepatitis C virus (HCV) infection."

Of the seven patients treated, three responded positively to treatment with Enbrel. In the Peterson et al. paper, the authors showed that several patients had significant improvements in viral load after TNF blocker or

treatment, but Peterson et al. did not call attention to this since they focused on the fact that there was no statistical significance between groups and they were looking for an exacerbation of hepatitis signal. However, both the Peterson data and the data shown in the present declaration indicate that there is a subset of patients who are responsive to TNF blocker therapy.

Table 1, attached hereto, describes the HCV RNA, PCR, AST, and ALT in the three patients with rheumatoid arthritis and Hepatitis C who responded to treatment with Etanercept.

Other workers in this field have since demonstrated that, for some cases, TNF blockers are effective in treating hepatitis:

1. Ohta et al., in *J Immunol* 2000 **165(2)**: 956-961 reported that in one animal model of hepatitis B, TNF blockers attenuated the hepatitis.
2. Mookerjee et al., *Gut* 2003 **52(8)**:1182-1187, reported on a study that showed that anti-TNF-alpha treatment in alcoholic hepatitis patients produces a highly significant, early, and sustained reduction in hepatic venous pressure gradient. In addition, there was a reduction in

hepatic inflammation and improved organ blood flow.

3. Tilg et al., in *J. Hepatol* 2003 34(4): 419-425, reported that patients with severe alcoholic hepatitis treated with a single infusion of infliximab. There was an early, though not significant, decrease in plasma levels of proinflammatory cytokines (IL-1beta, IL-6, IL-8). While TNF alpha expression did not change, expression of IL-8, a cytokine regulated mainly by TNF alpha, was almost absent on day +28.

4. Spahr et al., *J Hepatol* 2002 37(4):448-455, report that twenty patients with alcoholic hepatitis were treated with infliximab combined with steroids. The conclusion from this study was that, in severe alcoholic hepatitis, infliximab was well tolerated and associated with significant improvement in Maddrey's score at day 28.

It is clear from the above that some patients infected with a hepatitis virus respond positively to treatment with a TNF blocker.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By _____
Steven B. Abramson, M.D.

Date: 2/25/04

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Table 1 HCV RNA by PCR, AST, and ALT in Patients with Rheumatoid Arthritis and Chronic Hepatitis C before and after Treatment with Etanercept

	HCV RNA, PCR (copies/ml)	**AST** (units/L)	**ALT** (units/L)	**Liver Biopsy**	**Genotype**
Patient 1				1997- chronic active hepatitis 1999- chronic hepatitis with bridging fibrosis and nodule and formation, moderate steatosis	1b
1/00	985,000	137	116		
3/00		100	102		
	>1,000,000	100-200	100-200		
5/00 Etanercept started					
7/00	165,000	177	124		
9/00	121,000	48	53		
11/00	130,000	156	171		
Etanercept discontinued					
Patient 2				1993- mild nonspecific lymphocytic portal inflammation and minimal fatty change	?
11/96	366,000	58	39		
12/98	241,000	68	37		
12/98 Etanercept started					
2/99	211,000	N/A	36		
5/99	2829	26	36		
8/99	798	38	36		
9/99	989	34	38		
Etanercept discontinued by patient					
12/99	>1,000,000	37	40		
2/00	>1,000,000	44	38		
Patient 3				1994- chronic hepatitis C	1b
12/98	2733	34	28		
12/98 Etanercept started					
1/99	27.7	38	40		
5/99	37	37	35		
6/99 Etanercept discontinued due to rash					
7/00	detected		38		

1: J Rheumatol. 2004 Jan;31(1):107-9.

Related Articles, Links

Infliximab therapy for rheumatic diseases in patients with chronic hepatitis B or C.

Oniankitan O, Duvoux C, Challine D, Mallat A, Chevalier X, Pawlotsky JM, Claudepierre P.

Department of Rheumatology, Henri Mondor Teaching Hospital, AP-HP, Creteil, France.

OBJECTIVE: To describe the safety of tumor necrosis factor-a blockade in 2 patients with inflammatory rheumatic disease with chronic hepatitis B and C. METHODS: We used infliximab therapy in 2 patients with chronic inflammatory joint disease and chronic hepatitis B or C. We describe the clinical and laboratory test data obtained in these patients during the first year of treatment. Disease activity, liver function tests, and HCV and HBV status were evaluated before infliximab therapy was started and were reevaluated before each infusion. Liver biopsy was performed in both patients before infliximab therapy. RESULT: After more than one year of treatment, no worsening in liver function or virological status was observed, while a dramatic clinical improvement of joint disease was observed in both patients. CONCLUSION: These cases suggest that infliximab therapy may be safe in some quiescent or controlled chronic HBV or HCV infection.

PMID: 14705228 [PubMed - in process]

☐ 1: Eur J Gastroenterol Hepatol. 2001 Feb;13(2):191-2. Related Articles, Links

Infliximab therapy for Crohn's disease in the presence of chronic hepatitis C infection.

Campbell S, Ghosh S.

Gastrointestinal Unit, Western General Hospital, Edinburgh, UK. simoncampbell@hotmail.com

Treatment of Crohn's disease with infliximab is an important drug therapy for patients with refractory and fistulating disease. There are concerns over its use in a proportion of Crohn's patients with concurrent hepatitis C infection, since there are theoretical risks of accelerated hepatic decompensation due to the immunomodulatory impact of infliximab. We report a patient with both Crohn's disease and ongoing active hepatitis C infection who underwent infliximab therapy, with no worsening of his liver function or PCR status.

Publication Types:
- Case Reports

PMID: 11246620 [PubMed - indexed for MEDLINE]

1: J Dermatolog Treat. 2003 Dec;14(4):229-32. Related Articles, Links

Etanercept therapy in patients with autoimmunity and hepatitis C.

Khanna M, Shirodkar MA, Gottlieb AB.

Department of Medicine and Clinical Research Center, UMDNJ-Robert Wood Johnson Medical School, 51 French Street, New Brunswick, NJ 08901-0019, USA.

Accumulated data suggest that etanercept may be a therapeutic option in patients with hepatitis C and coexisting autoimmune disorders such as rheumatoid arthritis, psoriasis, psoriatic arthritis and ankylosing spondylitis. Additionally, etanercept may actually be of benefit, when used in combination with standard treatments, for hepatitis C.

Publication Types:
- Review
- Review, Tutorial

PMID: 14660270 [PubMed - indexed for MEDLINE]

Ann Rheum Dis. 2003 Nov;62(11):1078-82. Related Articles, Links

Effect of tumour necrosis factor alpha antagonists on serum transaminases and viraemia in patients with rheumatoid arthritis and chronic hepatitis C infection.

Peterson JR, Hsu FC, Simkin PA, Wener MH.

Division of Rheumatology, University of Washington, Seattle, 98195, USA.

BACKGROUND: Tumour necrosis factor alpha (TNF alpha) antagonists are effective for the treatment of rheumatoid arthritis (RA), but concerns remain about the safety of these agents in the presence of chronic infections, including hepatitis C virus (HCV) infection. OBJECTIVE: To examine the influence of treatment with TNF alpha antagonists on levels of HCV viraemia and serum transaminases in patients with RA and HCV. METHODS: In a retrospective survey the course of 16 HCV infected patients with RA who had received the TNF alpha antagonists etanercept or infliximab was analysed. Eight additional patients with RA and HCV were also enrolled into a three month prospective trial of etanercept. Serum concentrations of albumin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and HCV were followed. RESULTS: Viraemia was measured in 22 patients receiving a TNF alpha antagonist at the start of treatment and after 1-34 months (median 9 months follow up). Twenty four patients had serial tests of liver related enzymes and albumin. None of the differences between liver related tests at baseline and at follow up achieved significance ($p>0.05$). Similarly, the mean HCV measurement at 1-3, 4-6, 7-12, and 13-34 months did not differ significantly from baseline ($p>0.05$). CONCLUSION: In this study, liver related blood tests and HCV viral load measurements did not change substantially. These findings suggest that TNF alpha antagonists merit further study for the treatment of RA in HCV infected patients. Larger and longer term studies are still needed

From Discussion:
"TNFα antagonists are effective in the treatment of RA and have no known direct liver toxicity. Although there are concerns about their potential for exacerbation of infections,[20,21] including reactivation of tuberculosis,[22] the effect of TNFα antagonists on the course of HCV infection is not established.."

1: J Hepatol. 2002 Oct;37(4):448-55. Related Articles, Links

ELSEVIER FULL-TEXT ARTICLE

Combination of steroids with infliximab or placebo in severe alcoholic hepatitis: a randomized controlled pilot study.

Spahr L, Rubbia-Brandt L, Frossard JL, Giostra E, Rougemont AL, Pugin J, Fischer M, Egger H, Hadengue A.

Gastroenterology and Hepatology, University Hospital, 24 Rue Micheli-du-Crest, 1211, Geneva, Switzerland. laurent.spahr@hcuge.ch

BACKGROUND/AIMS: The aim of this study is to evaluate the tolerance and effects of infliximab combined with steroids in severe alcoholic hepatitis (AH). METHODS: Twenty patients with biopsy-proven severe AH (Maddrey's score>32) received prednisone 40 mg/day for 28 days and either infliximab 5mg/kg IV (group A) or placebo (group B) at day 0. Histology, plasma interleukin-6 (IL-6) and interleukin-8 (IL-8) were measured at baseline and at day 10. RESULTS: Infliximab was well tolerated. Histology showed no significant changes. At day 28, Maddrey's score significantly improved in group A (39 (32-53) to 12 (7-52), P<0.05 vs. baseline) but not in group B (44 (33-50) to 22 (2-59), P=NS). At day 10, IL-6 and IL-8 decreased in group A (25 pg/ml (10-85 pg/ml) to 4.5 pg/ml (2-25 pg/ml); 301 pg/ml (107-1207 pg/ml) to 14 6 pg/ml (25-252 pg/ml), P<0.01, P<0.05 vs. baseline, respectively). In group B, changes were not significant (38 pg/ml (13-116 pg/ml) to 16 pg/ml (4-128); 315 pg/ml (26-1698 pg/ml) to 110 pg/ml (27-492 pg/ml)). CONCLUSIONS: In severe AH, infliximab was well tolerated and associated with significant improvement in Maddrey's score at day 28. Although the size of this study does not allow comparison between groups, these promising results should encourage larger trials assessing the effects of this therapy on survival.

Publication Types:
- Clinical Trial
- Randomized Controlled Trial

PMID: 12217597 [PubMed - indexed for MEDLINE]

1: J Hepatol. 2003 Apr;38(4):419-25. Related Articles, Links

Comment on:
- J Hepatol. 2003 Apr;38(4):518-20.

ELSEVIER FULL-TEXT ARTICLE

Anti-tumor necrosis factor-alpha monoclonal antibody therapy in severe alcoholic hepatitis.

Tilg H, Jalan R, Kaser A, Davies NA, Offner FA, Hodges SJ, Ludwiczek O, Shawcross D, Zoller H, Alisa A, Mookerjee RP, Graziadei I, Datz C, Trauner M, Schuppan D, Obrist P, Vogel W, Williams R.

Department of Medicine, Division of Gastroenterology and Hepatology, University Hospital Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria. herbert.tilg@uibk.ac.at

BACKGROUND/AIMS: Severe alcoholic hepatitis (AH) is associated with high mortality. Tumor necrosis factor-alpha (TNFalpha) has been demonstrated to play an important role in its pathophysiology. METHODS: Twelve patients with biopsy-confirmed AH and a Maddrey discriminant factor >32 were treated with a single infusion of the anti-TNF monoclonal antibody Infliximab at a dose of 5mg/kg body weight. Serial measurements were made for various cytokines using specific enzyme-linked immunoassays (ELISA). In four patients, liver biopsy samples were available pretreatment and on day+28 of therapy. RESULTS: Ten of the 12 patients are alive at a median of 15 (12-20) months. Two patients died within 30 days from septicemia. Serum bilirubin levels, Maddrey score, neutrophil count and C-reactive protein fell significantly within the first month. There was an early, though not significant, decrease in plasma levels of proinflammatory cytokines (interleukins (IL)-1beta, IL-6, IL-8, interferon-gamma), whereas plasma levels of TNFalpha remained near the sensitivity limit of the assay throughout the treatment course. While TNFalpha mRNA expression in the liver did not change, expression of IL-8, a cytokine regulated mainly by TNFalpha, was almost absent on day+28. CONCLUSIONS: Our data suggest that randomized controlled trials of anti-TNF antibody in severe AH are warranted.

Publication Types:
- Clinical Trial
- Comment
- Multicenter Study

1: Gut. 2003 Aug;52(8):1182-7.

Tumour necrosis factor alpha is an important mediator of portal and systemic haemodynamic derangements in alcoholic hepatitis.

Mookerjee RP, Sen S, Davies NA, Hodges SJ, Williams R, Jalan R.

Liver Failure Group, Institute of Hepatology, London, UK.

BACKGROUND: The role of proinflammatory cytokines in the pathogenesis of portal hypertension is unclear. AIMS AND METHODS: This study tests the hypothesis that tumour necrosis factor alpha (TNF-alpha) is an important mediator of the circulatory disturbances in alcoholic hepatitis (AH) and evaluates the acute and short term effect of a single infusion of the monoclonal chimeric anti-TNF-alpha antibody (Infliximab) on portal and systemic haemodynamics in 10 patients with severe biopsy proven AH. Cardiovascular haemodynamics, hepatic venous pressure gradient (HVPG), and hepatic and renal blood flow were measured before, 24 hours after Infliximab, and prior to hospital discharge. RESULTS: Serum bilirubin ($p<0.05$), C reactive protein ($p<0.001$), and white cell count ($p<0.01$) were reduced significantly, as were plasma levels of interleukin (IL)-6 and IL-8 after treatment. Of the 10 patients, nine were alive at 28 days. Mean HVPG decreased significantly at 24 hours (23.4 (2.8) to 14.3 (1.9) mm Hg; $p<0.001$) with a sustained reduction prior to discharge (12.8 (1.9) mm Hg; $p<0.001$). Mean arterial pressure and systemic vascular resistance increased significantly ($p<0.001$and $p<0.01$, respectively), mirrored by a reduction in cardiac index (5.9 (0.5) to 4.7 (0.5) l/min/m(2); $p<0.05$) prior to discharge. Hepatic and renal blood flow also increased significantly (506.2 (42.9) to 646.3 (49.2) ml/min ($p=0.001$) and 424.3 (65.12) to 506.3 (85.7) ml/min ($p=0.001$), respectively) prior to discharge. CONCLUSION: The results of this study illustrate that anti-TNF-alpha treatment in AH patients produces a highly significant, early, and sustained reduction in HVPG, possibly through a combination of a reduction in cardiac output and intrahepatic resistance. In addition, there was a reduction in hepatic inflammation and improved organ blood flow, suggesting an important role for TNF-alpha in mediating the circulatory disturbances in AH.

PMID: 12865279 [PubMed - indexed for MEDLINE]